**Vaginal high-risk human papillomavirus infection in a cross-sectional study among women of six different ethnicities in Amsterdam, the Netherlands: the HELIUS study**

C.J. Alberts MSc­­1,2,

R. A. Vos MSc1,

H. Borgdorff MD 2,3,

W. Vermeulen1,

Prof dr. J. van Bergen MD4,5,

S. Bruisten PhD1,2,

Prof dr. S.E. Geerlings2 MD,

M.B. Snijder PhD6,

R. van Houdt MSc PhD7,

Prof dr. S. Morré8,9,

Prof dr. H. J.C. de Vries MD2,10,

Prof dr. J. van de Wijgert 2,3,11,

Prof dr. M. Prins1,2,

Dr. M.F. Schim van der Loeff MD1,2

**1.** Department of Infectious Diseases, Public Health Service of Amsterdam (GGD), Amsterdam, the Netherlands

**2.** Department of Internal Medicine, Division of Infectious Diseases, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center (AMC), Amsterdam, the Netherlands

**3.** Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, the Netherlands

**4.** SOA Aids Nederland, Amsterdam, the Netherlands

**5.** Department of General Practice, Academic Medical Center (AMC), Amsterdam, the Netherlands

**6.** Department of Public Health, Academic Medical Center (AMC), Amsterdam, the Netherlands

**7.** Department of Medical Microbiology and Infection Control, VU Medical Center, Amsterdam the Netherlands

**8.** Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control, Research School V-ICI, VU University Medical Center, Amsterdam, the Netherlands.
**9.** Institute for Public Health Genomics, Department of Genetics and Cell Biology, Research Institute  GROW (School for Oncology & Developmental Biology), Faculty of Health, Medicine & Life Sciences, University of Maastricht,  Maastricht, The Netherlands  **10.** Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands **11.** Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

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**Corresponding author:**

Catharina J. Alberts, Public Health Service, Department of Infectious Diseases,

Nieuwe Achtergracht 100, 1018 WT, Amsterdam, the Netherlands.

E-mail: nalberts@ggd.amsterdam.nl

Telephone +31 (0)20 555 5083, fax +31 (0)20 555 5533

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**ABSTRACT**

**Objective**

In the Netherlands the incidence of cervical cancer is higher among ethnic minority populations compared to the general Dutch population. We investigated the prevalence of, and risk factors associated with, vaginal hrHPV infection in women of six different ethnicities living in Amsterdam.

**Methods**

For this cross-sectional study we selected women aged 18-34 years old of six ethnicities from the large-scale multi-ethnic HELIUS study. Self-collected vaginal-swabs were tested for HPV DNA and genotyped using a highly sensitive PCR and reverse line blot assay (SPF10-PCR-DEIA/LiPA25-system version-1,DDL). Participants completed a questionnaire regarding demographics and sexual behaviour. Logistic regression using generalized estimating equations was used to assess risk factors of hrHPV, and to investigate whether prevalence of hrHPV differed among ethnicities.

**Results**

The study population consisted of 592 women with a median age of 27 (IQR: 23-31) years. Dutch and African Surinamese women reported the highest sexual risk behaviour. HrHPV prevalence was highest in the Dutch (40%) followed by the African Surinamese (32%), Turkish (29%), Ghanaian (26%), Moroccan (26%), and South-Asian Surinamese (18%). When correcting for sexual risk behaviour, the odds to be hrHPV positive were similar for all non-Dutch groups when compared to that of the Dutch group.

**Conclusion**

We found an overall higher hrHPV prevalence and higher sexual risk behaviour in the native Dutch population. Further research is needed to unravel the complex problem concerning cervical cancer disparities, such as differences in participation in the cervical cancer screening program, or differences in clearance and persistence of hrHPV.

**Key messages:**

- We investigated the prevalence of, and risk factors associated with, vaginal high-risk HPV infection in different ethnicities in Amsterdam.

- High-risk HPV prevalence and sexual risk behaviour was overall lower among women of non-Dutch ethnicities compared to Dutch women.

- Further research is needed to explain cervical cancer disparities among ethnic minorities.

**MeSH terms:** (1) papillomaviridae, (2) Netherlands, (3) women, (4) health status disparities, (5) epidemiology, (6) cross-sectional studies

**INTRODUCTION**

Cervical cancer is the fourth most common female cancer globally; the majority of this burden is in non-western regions1. When women from those regions immigrate to the west, they still show higher cervical cancer incidence and related mortality when compared to the native western population2 3, despite the fact that the same health care is offered to all women. In the Netherlands, these health disparities are also observed4 5: women born in Morocco, Surinam, Aruba/Netherlands Antilles, and Indonesia have a higher cervical cancer incidence compared to the Dutch population. Possible reasons for these differences, which are not mutually exclusive, are (a) differences in (sexual) risk behaviour6-9; (b) differences in participation in the cervical cancer screening program10 11; (c) differences in high-risk human papillomavirus (hrHPV) prevalence12, the distribution of hrHPV types13 14 or clearance of hrHPV15; and/or (d) a reflection of the higher incidence of cervical cancer in their home country 5 16.

In the Netherlands approximately 12% of the population is of non-western origin and this group is expected to grow in the coming years17. Data on prevalence and determinants of HPV infection among these groups are scarce and the few studies conducted lack the power to show associations18-20. Our objective was to map the prevalence of hrHPV infection among ethnic minorities and Dutch women aged 18-34 years. In this way we hope to provide insight into why the incidence of cervical cancer is higher among ethnic minorities compared to the Dutch. Therefore, we selected from the multi-ethnic population-based HELIUS study women with a Dutch, South-Asian Surinamese, African Surinamese, Ghanaian, Moroccan, or Turkish origin living in Amsterdam, the Netherlands. By assessing the prevalence of vaginal hrHPV infections in these women and relating this to their sexual behaviour and other characteristics, we address in this cross-sectional study the following questions: (1) What is the reported sexual risk behaviour? (2) What is the prevalence of hrHPV? (3) Which hrHPV types are most common? (4) What are risk factors for being hrHPV positive? (5) What is the trend of hrHPV prevalence by age? and (6) Does the prevalence of hrHPV infections differ between the six ethnic groups after adjustment for important risk factors?

**METHODS**

**Study population**

Female participants were selected from the HELIUS (HEalthy LIfe in an Urban Setting) study, a large-scale multi-ethnic cohort study being carried out in Amsterdam, the Netherlands21. In brief, participants of Dutch, Surinamese, Ghanaian, Moroccan and Turkish origin aged 18 and 70 years were randomly selected from the municipality registry of Amsterdam. Of the Surinamese immigrants in the Netherlands, approximately 80% are of either African origin (from West-Africa) or South-Asian origin (from North India), and the remaining 20% are of Javanese, Chinese, or other origin. Surinamese subgroups were classified according to self-reported ethnic origin. In this particular study, the South-Asian Surinamese group also included participants of Javanese origin. A participant was considered as of non-Dutch ethnic origin if she fulfilled one of the following criteria22: (a) born outside the Netherlands and has at least one parent who was born outside the Netherlands (1st generation) or (b) born in the Netherlands and at least one of the parents was born outside of the Netherlands (2nd generation). The study was approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam (protocol number:10/100; amendment10/100# 10.17.1729; NL32251.018.10).

At the time of this study, recruitment of participants into HELIUS was still on-going. We selected participants recruited from January 2011 through December 2013. We randomly selected up to seven (if available) female participants per life year, in the age range of 18 to 34 years (=17 years), from each ethnic group, resulting in a maximum of 119 (=7x17) participants per ethnic group. Participants were eligible if they provided a vaginal sample and completed questions on their sexual behaviour. Participants who indicated to be vaccinated with a prophylactic HPV vaccine were excluded.

**Data and sample collection**

Detailed information regarding socio-demographic characteristics (age, education, civil status, religion, self-identified ethnicity in Surinamese participants), health-related characteristics (HPV vaccination status, ever used oral contraceptives and self-reported genital warts/ulcers/vesicles in the past), substance use (smoking and alcohol use), and sexual behaviour (ever had sex, currently in a steady relationship, age at sexual debut, number of lifetime male sex partners (LMSP), sexual activity in the preceding six months, and condom use in preceding six months during sex with a steady or casual partner) were obtained via standardized questionnaires. All female participants were asked to self-collect a vaginal swab (Copan Diagnostics, Inc., Murrieta, CA, USA).

**HPV testing**

The self-collected vaginal swabs were kept at 2-8ºC for up to 5-6 days, shipped to the Public Health Laboratory and then stored at -20ºC. Selected swabs for this study were tested for *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhoeae* (NG) (Aptima Combo 2 for CT/NG assay and the Aptima *Trichomonas vaginalis* assay by PANTHER System, HOLOGIC (Bedford, USA)) and tested for HPV (SPF10-PCR-DEIA/LiPA25 system version-1, DDL, Voorburg, the Netherlands) according to protocol and as described earlier 23 24. HPV types were categorized as indicated in earlier studies 24 25.

**Statistical analyses**

Pearson’s Chi-squared test was used to compare categorical data and Kruskal-Wallis test to compare continuous data across ethnic groups. The main outcome of interest was hrHPV positivity. Age and sexual debut were categorized based on quartiles and LMSP was categorized based on risk categories of interest. For logistic regression analyses, Generalized Estimating Equation (GEE) models with exchangeable correlation structure were used to account for multiple HPV infection within the same participant26; GEE models take account of clustered data as one participant is at risk for different HPV types at the same time. Statistical analyses were performed using Stata software version 13.1 (Stata Intercooled, College Station,TX,USA).

*Risk factor analyses and hrHPV by age*

Bivariable logistic regression (using GEE) was used to investigate risk factors associated with hrHPV in each ethnic group. Subsequently, we assessed in an overall model whether these risk factors for hrHPV positivity differed between ethnicities by testing whether the interaction between ethnicity and the individual risk factors was statistically significant (p<0.05). We estimated the probability of hrHPV infections as a function of age using a restricted cubic spline with 4 knots that was allowed to differ by ethnic group.

*Association between high-risk HPV and ethnicity*

To study the association between hrHPV and ethnicity we conducted multivariable logistic regression analyses (using GEE). We controlled for risk factors by including a priori defined variables. We ran six different models. Model 1, 2, and 3 included all 592 female participants of the study population and model 4, 5, and 6 were restricted to 476 out of the 592 female participants who reported to ever have had sex with a man. The reason to perform separate analyses for those who reported to ever have had sex with a man is to enable adjusting for more than one sexual risk variable. Models 1 and 4 show the crude association between hrHPV and ethnicity; models 2 and 5 are adjusted for age, education, and civil status; model 3 is adjusted for age, education, civil status and LMSP; while model 6 is adjusted for age, education, civil status, age of sexual debut, LMSP, and sexual activity in the preceding six months. Risk factors were chosen to adjust for (behavioural) differences between ethnicities.

**RESULTS**

*Participants versus non-participants*

During the period of January 2011 through December 2013, a total of 10,257 participants were enrolled in the HELIUS study; 1,469 of these participants were women aged 18-34 years. In total 980 women aged 18-34 years agreed to provide a vaginal sample and 489 declined (Supplementary figure 1). Dutch (94%), African Surinamese (87%), South-Asian Surinamese (75%) and Ghanaian (71%) provided more often a vaginal swab compared to Moroccan (49%) and Turkish (37%) women (*p*<0.001). Turkish and Moroccan women who declined to provide a vaginal sample reported higher sexual risk behaviour than those who did provide a vaginal swab (data not shown).

*Study population*

Of the 980 eligible female participants with a vaginal sample available, 610 were randomly selected. From those selected we excluded 10 participants because they indicated to have been vaccinated against HPV and 8 participants because of invalid HPV results (Supplementary figure 1). Baseline characteristics of the 592 included participants stratified by ethnicity are shown in Table 1. Overall, the median age was 27 years (interquartile range [IQR]:23-31) and was similar between ethnic groups (*p*=0.353). Most characteristics differed significantly between the ethnic groups. For example, women of Dutch origin more often had had a higher education (70%), were non-religious (92%), ever used oral contraceptives (89%), and used alcohol in the preceding year (94%) when compared to other ethnicities. STI diagnosed at visit did not differ significantly (*p*=0.070) between ethnicities, however the highest prevalence was found in African Surinamese women (8% STI positive, mainly CT). Almost all Dutch (95%) and African Surinamese (93%) reported to ever have had sex, whereas in the South-Asian Surinamese (82%), Ghanaian (69%), Moroccan (65%), and Turkish (77%) group, fewer indicated to ever had sex with a man or a woman (*p*<0.001). Among those who reported to ever have had sex, sexual behaviour differed significantly between ethnicities: e.g. Dutch and African Surinamese women reported a younger sexual debut, a higher number of LMSP, and more often having sex with a casual partner or with both a casual and steady partner, in comparison to women of other ethnicities.

**HPV prevalence**

HPV prevalence by ethnicity is presented in Table 2 and a graphical representation of the distribution of the individual hrHPV types by ethnicity is presented in Figure 1.a. In total 280/592 (47%) of samples from women were PCR-DEIA positive, of which 42 were untypable. AnyHPV (53%), hrHPV (42%), and lrHPV (31%) prevalence were highest among the Dutch. HPV types 16 and 18 were detected among all groups, but were most common among Turkish (HPV-16; 13%) and Dutch (HPV-18; 10%) women respectively. The prevalence of individual hrHPV types did not differ significantly between ethnicities except for HPV type 18 (*p*=0.006).

**Risk factor analyses and hrHPV by age**

We did not find any socio-demographic characteristics, health related characteristics or substance use characteristics that were significantly associated with hrHPV positivity in all ethnicities (for details see Supplementary Table 1 and 2). HrHPV positivity was significantly associated with a higher number LMSP in the Dutch (*p*=0.006), South-Asian Surinamese (*p*=0.005), Moroccan (*p*=0.001) and Turkish (*p*=0.002) groups. Odds for hrHPV positivity was the highest among those reporting sex with a casual only and a casual and steady partner, but this was only statistically significant in the Dutch, South-Asian Surinamese and Turkish groups. No other sexual risk variables were found to be associated in almost all ethnic groups. No statistically significant interactions (all *p*>0.05) between ethnicity and any of the risk factors under investigation were found (data not shown). Figure 1.b shows hrHPV prevalence by age for each ethnicity. Prevalence of hrHPV was highest around the age of 25-30 and lower in higher ages; this trend did not differ significantly between ethnicities (*p*=0.796).

**Association between high-risk HPV and ethnicity**

Table 3 shows the association between ethnicity and hrHPV using GEE. In the full population (n=592) we observed that African Surinamese did not significantly differ from Dutch women, whereas South-Asian Surinamese, Ghanaian, and Moroccan women had a lower odds to be hrHPV positive than Dutch women. When adjusting for non-sexual behavioural characteristics (i.e. age, education, and civil status) similar results were obtained, however the odds for African Surinamese women to be hrHPV positive were now significantly lower when compared to the Dutch women (model 2). When we also adjusted for number of LMSP the differences between ethnicities were no longer significant (model 3). When restricting the analyses to female participants who reported to ever have had sex with a man, we observed similar results as in the full study population.

**Discussion**

In the Netherlands, incidence of cervical cancer is reported to be higher among ethnic minorities when compared to the general population4 5. These differences might be caused for example by differences in prevalence of hrHPV infections or sexual behaviour. We found that among this cohort of young women aged 18-34 years, both hrHPV prevalence and sexual risk behaviour were overall highest in women with a Dutch origin. Furthermore, we did not find indications that the effects of risk factors for hrHPV differed significantly between ethnicities. To further understand the complex web of factors underlying the differences in cervical cancer incidence, we formulated six key questions that the data allowed us to address.

The first two questions were: (1) What is the sexual risk behaviour reported? and, (2) What is the prevalence of hrHPV? We found that sexual risk behaviour differed significantly between ethnicities with the Dutch and African Surinamese groups reporting the highest sexual risk behaviour. The hrHPV prevalence was highest in the Dutch group, followed by the non-Dutch groups. We did not expect that the groups at highest risk for cervical cancer would show the lowest overall hrHPV prevalence. There are several possible interpretations for these results. For instance, the prevalences of hrHPV in the current (younger) study population may be different from those in the women in which differences in cervical cancer incidence by ethnicity were observed in the past (older age group), and therefore perhaps may not explain the disparities observed among these ethnicities. Another explanation could be that other factors play a role in the development of cervical cancer, for example differences in participation in cervical cancer screening, higher exposure at later age, or differences in clearance and persistence of hrHPV.

Next, we assessed which hrHPV types were most common in the six ethnic groups (question 3). We found that the prevalence of individual hrHPV types did not differ significantly between ethnicities, except for HPV-18. Although the prevalences of other hrHPV types were not significantly different, Figure 1.a suggests that among women with African ancestors (African Surinamese, Ghanaian, and Moroccan) HPV-16 and HPV-18 do not comprise the largest fraction of all detected types, as suggested previously13 14. It is therefore worthwhile considering whether the recently FDA approved 9-valent vaccine27 28 would be an asset in the efforts to control cervical cancer in Dutch ethnic minorities. However, a recent study from the US found that only a small additional reduction in HPV-associated cancer cases is expected when using the new 9-valent vaccine across all ethnicities; nevertheless in some ethnic groups a larger additional reduction is expected for cervical cancer in situ and oropharyngeal cancers29.

What are the risk factors for being hrHPV positive (question 4)? Our findings are in line with previous studies6 7 30; sexual risk behavioural characteristics are associated with hrHPV positivity. However, in contrast to previous studies8 31, we did not observe a clear association between hrHPV positivity and age of sexual debut in any of the ethnicities, which may be caused by lack of power. We also did not find indications that the effects of risk factors for hrHPV or the trend in hrHPV positivity by age (question 5) differed significantly between ethnicities.

The sixth and last question is whether the prevalence of hrHPV infections differed between the six ethnic groups after adjustment for important risk factors. We conclude that differences in hrHPV positivity are caused by differences in sexual risk behaviour between ethnicities. However, we also observe that among Moroccan and Turkish women the odds ratio changed from smaller than one to larger than one. This change of direction is due to the fact that Moroccan and Turkish women with low sexual risk behaviour have higher hrHPV prevalences than Dutch women with similar reported sexual risk behaviour. There are three possible reasons for this higher prevalence: (i) Moroccan and Turkish women have a higher risk to become hrHPV positive than Dutch women when practising the same sexual behaviour, (ii) Moroccan and Turkish women more often have non-penetrative sex when reporting to never have had sex, or (iii) social desirability bias may differ between ethnicities.

This study has some limitations. Although HELIUS participants were recruited from the general population of Amsterdam, and our study population was randomly selected from the HELIUS population, there may be selection bias in our study population. First, participants of the HELIUS study may be more concerned about their health, and secondly, less than half of the Turkish and Moroccan HELIUS participants agreed to provide a vaginal swab. Therefore, we compared our results on sexual behaviour with the national Dutch Public Health database32 and observed similar patterns of reported sexual risk behaviours. Another limitation of this study is the cross-sectional nature that prevents investigation of differences in incidence and persistence of hrHPV infection.

In our study in Amsterdam we found higher hrHPV prevalences than observed in previous studies in other parts of the Netherlands with a hrHPV prevalence of 12% (ages 18-29 years)30 and 11% (ages 18-34 years)33. A self-sampling study investigating hrHPV prevalence in various ethnic groups showed prevalences of 9% in ethnic-Dutch, 11% in the Surinamese, 8% in Moroccan, and 8% in Turkish women, respectively20. This suggests that our population concerns a higher risk group.

The ethnic minority populations in the Netherlands, and especially in large cities such as Amsterdam, are increasing. Here we report that hrHPV prevalence and sexual risk behaviour were highest in the Dutch group, indicating that other factors must be responsible for the higher incidence of cervical cancer in ethnic minorities. These findings emphasize the need for further research to unravel the complex problem concerning cervical cancer disparities such as differences in participation in the cervical cancer screening program or differences in clearance and persistence of high-risk human papillomavirus (hrHPV) prevalence.

**Conflict of Interest Statement**

M. F. Schim van der Loeff received research funding from Sanofi Pasteur MSD; he is a co-investigator in a Merck-funded investigator-initiated study on Gardasil; he is an investigator on a Sanofi Pasteur MSD sponsored HPV vaccine trial; he served on a vaccine advisory board of GSK; he received in-kind contribution for another study from Stichting Pathologie Onderzoek en Ontwikkeling (SPOO); his institution receives research funding from Janssen Infectious Diseases and Vaccines; other authors: no conflicts of interest.

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Author contributions

MBS and MP conceived and designed the HELIUS study and obtained funding. WV performed laboratory analyses of the samples. WV, CJA, RAV and HB organised sample logistics. CJA and HB participated in data collection. All authors participated in designing the vaginal swab study within HELIUS and/or laboratory assay development. CJA and RAV performed datamanagment and statistical analyses. CJA wrote manuscript. MFSvdL (principal investigator) contributed to the design, statistical analyses and writing the manuscript. All authors contributed to data interpretation, reviewed successive drafts and approved the final version of the manuscript.

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| **Table 1.** Baseline characteristics of 592 female HELIUS participants aged between 18 and 34 years who provided a vaginal swab and completed the questionnaire, by ethnicity, the HELIUS study Amsterdam, the Netherlands |
|  | **Dutch** | **South-Asian** | **African** | **Ghanaian** |  **Moroccan** | **Turkish** |  |  **Total** |
|  | **Surinamese** | **Surinamese** |  |
|  | (N=108) | (N=100) | (N=111) | (N=81) | (N=103) | (N=89) |  *p-value* | (N=592) |
|   | n | % | n | % | n | % | n | % | n | % | n | % |   | n | % |
| **Socio-demographic characteristics** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Median age in years (IQR) | 27 (22 - 31) | 27 (23 - 31) | 26 (22 - 31) | 25 (21- 30) | 27 (23- 31) | 27 (23- 31) | 0.353 | 27 (23- 31) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Age (years) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  18-23 | 34 | 31% | 28 | 28% | 36 | 32% | 35 | 43% | 28 | 27% | 24 | 27% | 0.904 | 185 | 31% |
|  24-27 | 25 | 23% | 25 | 25% | 26 | 23% | 16 | 20% | 28 | 27% | 21 | 24% |  | 141 | 24% |
|  28-30 | 21 | 19% | 21 | 21% | 21 | 19% | 12 | 15% | 21 | 20% | 21 | 24% |  | 117 | 20% |
|  31-34 | 28 | 26% | 26 | 26% | 28 | 25% | 18 | 22% | 26 | 25% | 23 | 26% |  | 149 | 25% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Education\* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  Lower and intermediate education | 32 | 30% | 65 | 65% | 76 | 69% | 63 | 79% | 67 | 65% | 66 | 74% | **<0.001** | 369 | 63% |
|  Higher education | 76 | 70% | 35 | 35% | 34 | 31% | 17 | 21% | 36 | 35% | 23 | 26% |  | 221 | 37% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Civil status |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  Married/cohabiting | 32 | 30% | 32 | 32% | 16 | 15% | 15 | 19% | 39 | 38% | 42 | 47% | **<0.001** | 176 | 30% |
|  Never married | 76 | 70% | 63 | 63% | 93 | 85% | 62 | 79% | 54 | 52% | 41 | 46% |  | 389 | 66% |
|  Divorced | 0 | 0% | 5 | 5% | 0 | 0% | 1 | 1% | 10 | 10% | 6 | 7% |  | 22 | 4% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Current religion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No religion | 99 | 92% | 23 | 23% | 33 | 30% | 7 | 9% | 3 | 3% | 13 | 15% | **<0.001** | 178 | 30% |
|  Roman Catholic Church | 3 | 3% | 5 | 5% | 34 | 31% | 11 | 14% | 0 | 0% | 0 | 0% |  | 53 | 9% |
|  Christianity (other than Roman Catholic)  | 4 | 4% | 5 | 5% | 38 | 34% | 58 | 73% | 0 | 0% | 3 | 3% |  | 108 | 18% |
|  Islam | 1 | 1% | 19 | 19% | 0 | 0% | 1 | 1% | 99 | 96% | 71 | 80% |  | 191 | 32% |
|  Hinduism | 0 | 0% | 42 | 42% | 0 | 0% | 1 | 1% | 0 | 0% | 0 | 0% |  | 43 | 7% |
|  Other | 1 | 1% | 6 | 6% | 6 | 5% | 2 | 3% | 1 | 1% | 2 | 2% |  | 18 | 3% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Migration status |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  1st generation | N.A. |  | 29 | 29% | 44 | 40% | 51 | 63% | 32 | 31% | 24 | 27% | **<0.001** | 180 | 37% |
|  2nd generation |  |  | 71 | 71% | 67 | 60% | 30 | 37% | 71 | 69% | 65 | 73% |  | 304 | 63% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Health-related characteristics** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Oral contraceptives use ever |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No | 12 | 11% | 21 | 21% | 14 | 13% | 38 | 47% | 33 | 32% | 20 | 22% | **<0.001** | 138 | 23% |
|  Yes | 96 | 89% | 79 | 79% | 97 | 87% | 43 | 53% | 70 | 68% | 69 | 78% |  | 454 | 77% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Self-reported genital warts/ulcers/vesicles in the preceding month |  |  |  |  |  |  |  |  |  |  |  |
|  No | 105 | 97% | 95 | 95% | 106 | 95% | 76 | 94% | 100 | 97% | 84 | 94% | 0.817 | 566 | 96% |
|  Yes | 3 | 3% | 5 | 5% | 5 | 5% | 5 | 6% | 3 | 3% | 5 | 6% |  | 26 | 4% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Substance use** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Smoking status |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  Never | 53 | 49% | 67 | 68% | 69 | 63% | 78 | 96% | 83 | 81% | 41 | 46% | **<0.001** | 391 | 66% |
|  Former | 22 | 20% | 10 | 10% | 12 | 11% | 3 | 4% | 6 | 6% | 12 | 13% |  | 65 | 11% |
|  Current | 33 | 31% | 22 | 22% | 29 | 26% | 0 | 0% | 14 | 14% | 36 | 40% |  | 134 | 23% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Alcohol use in preceding year |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No | 7 | 6% | 37 | 37% | 24 | 22% | 40 | 49% | 93 | 90% | 62 | 70% | **<0.001** | 263 | 45% |
|  Yes | 101 | 94% | 62 | 63% | 87 | 78% | 41 | 51% | 10 | 10% | 27 | 30% |  | 328 | 55% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **STI at visit\*\*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No | 105 | 97% | 99 | 99% | 102 | 92% | 75 | 93% | 99 | 96% | 87 | 98% | 0.070 | 567 | 96% |
|  Yes | 3 | 3% | 1 | 1% | 9 | 8% | 6 | 7% | 4 | 4% | 2 | 2% |  | 25 | 4% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Sexual behaviour** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sexual activity ever\*\*\* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  Never had sex  | 5 | 5% | 18 | 18% | 8 | 7% | 25 | 31% | 36 | 35% | 20 | 22% | **<0.001** | 112 | 19% |
|  Sex with a man (+/- woman) | 103 | 95% | 82 | 82% | 101 | 92% | 56 | 69% | 66 | 64% | 68 | 76% |  | 476 | 81% |
|  Sex with a woman only | 0 | 0% | 0 | 0% | 1 | 1% | 0 | 0% | 1 | 1% | 1 | 1% |  | 3 | 1% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Currently in a steady relationship |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No | 48 | 44% | 44 | 44% | 59 | 53% | 44 | 54% | 55 | 54% | 38 | 43% | 0.331 | 288 | 49% |
|  Yes | 60 | 56% | 56 | 56% | 52 | 47% | 37 | 46% | 47 | 46% | 50 | 57% |  | 302 | 51% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***Among participants reporting to ever have had sex with a man (n=476)*** |  |  |  |  |  |  |  |  |  |  |  |
| Median age at sexual debut in years (IQR) | 16 (15-18) | 18 (17-20) | 16 (15-18) | 18 (16-19) | 20 (18-22) | 20 (18-22) | **<0.001** | 18 (16-20) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Age at sexual debut (years) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  ≤16 | 56 | 54% | 18 | 22% | 52 | 51% | 17 | 30% | 12 | 18% | 5 | 7% | **<0.001** | 160 | 34% |
|  17-18 | 29 | 28% | 32 | 39% | 29 | 29% | 22 | 39% | 13 | 20% | 14 | 21% |  | 139 | 29% |
|  19-20 | 8 | 8% | 18 | 22% | 16 | 16% | 6 | 11% | 12 | 18% | 17 | 25% |  | 77 | 16% |
|  ≥21 | 10 | 10% | 14 | 17% | 4 | 4% | 11 | 20% | 29 | 44% | 32 | 47% |  | 100 | 21% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lifetime number of male sex partners |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  1 | 10 | 10% | 31 | 38% | 7 | 7% | 12 | 21% | 44 | 67% | 43 | 63% | **0.002** | 147 | 31% |
|  2-5 | 37 | 36% | 37 | 45% | 46 | 46% | 31 | 55% | 15 | 23% | 14 | 21% |  | 180 | 38% |
|  6-10 | 26 | 25% | 10 | 12% | 30 | 30% | 9 | 16% | 4 | 6% | 10 | 15% |  | 89 | 19% |
|  ≥11 | 30 | 29% | 4 | 5% | 18 | 18% | 4 | 7% | 3 | 5% | 1 | 1% |  | 60 | 13% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sexual activity in the preceding six months |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No sex in the preceding six months | 19 | 18% | 16 | 20% | 11 | 11% | 11 | 20% | 16 | 24% | 12 | 18% | **0.001** | 85 | 18% |
|  With steady partner only | 60 | 58% | 61 | 74% | 67 | 66% | 41 | 73% | 48 | 73% | 44 | 65% |  | 321 | 67% |
|  With casual partner(s) (+/-steady partner(s)) | 24 | 23% | 5 | 6% | 23 | 23% | 4 | 7% | 2 | 3% | 12 | 18% |  | 70 | 15% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Condom use in the preceding six months |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  No sex in the preceding six months | 19 | 18% | 16 | 20% | 11 | 11% | 11 | 20% | 16 | 25% | 12 | 18% | 0.416 | 85 | 18% |
|  Never/mostly not/sometimes | 63 | 61% | 55 | 67% | 63 | 62% | 32 | 57% | 38 | 58% | 44 | 65% |  | 295 | 62% |
|  Mostly/always | 21 | 20% | 11 | 13% | 27 | 27% | 13 | 23% | 11 | 17% | 12 | 18% |  | 95 | 20% |
| **Abbreviations:** HELIUS,HEalthy LIfe in Urban Setting; HPV, human papillomavirus; IQR=interquartile range; STI, sexually transmitted infection; N.A.=not applicable |
|  +/- denotes an optional category e.g. +/- steady partner indicates that individuals with and without a steady partners are included in this category |
| *p*-values <0.05 are considered statistically significant; categorical variables are based on Chi-squared test and *p*-values of continuous variables are based on the Kruskall-Wallis test |
| \* Education was categorized as lower and intermediate education if participant (1) never had been to school or had elementary schooling only, (2) followed vocational schooling or lower secondary schooling, or (3) followed intermediate/higher secondary education schooling; and was categorized as higher education if participant had followed higher vocational schooling or university. |
| \*\* Chlamydia, gonorrhoea, or trichomoniasis (nobody tested positive for gonorrhoea) |
| \*\*\* One participant reported to ever have had sex but did not report whether this was with a man or a woman |
| Data are missing for education (n=2), civil status (n=5), current religion (n=1), smoking status (n=2), alcohol use (n=1), currently in steady relationship (n=2), lifetime number of male sexual partners (n=1), condom use in the preceding six months (n=2) |
|  |

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| --- |
| **Table 2.** HPV prevalence in 592 randomly selected female HELIUS participants aged between 18 and 34 years who provided a vaginal swab and completed the questionnaire, by ethnicity, the HELIUS study Amsterdam, the Netherlands |
|  | **Dutch** | **South-Asian Surinamese** | **African Surinamese** | **Ghanaian** | **Moroccan** | **Turkish** |  | **Total** |
|  |  |
|  | (N=108) | (N=100) | (N=111) | (N=81) | (N=103) | (N=89) | *p*-value*\** | (N=592) |
|   | n | % | n | % | n | % | n | % | n | % | n | % |   |   | % |
| PCR-DEIA positive | 63 | 58% | 39 | 39% | 67 | 60% | 37 | 46% | 39 | 38% | 35 | 39% | **0.001** | 280 | 47% |
| Any HPV \*\* | 57 | 53% | 29 | 29% | 56 | 50% | 30 | 37% | 34 | 33% | 32 | 36% | **0.001** | 238 | 40% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Any high-risk HPV | 45 | 42% | 18 | 18% | 36 | 32% | 21 | 26% | 27 | 26% | 26 | 29% | **0.008** | 173 | 29% |
| ≥ 2 high risk HPV types | 22 | 20% | 5 | 5% | 13 | 12% | 3 | 4% | 8 | 8% | 10 | 11% | **0.005** | 61 | 10% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HPV 16 | 7 | 6% | 4 | 4% | 6 | 5% | 3 | 4% | 6 | 6% | 12 | 13% | 0.087 | 38 | 6% |
| HPV 18 | 11 | 10% | 4 | 4% | 3 | 3% | 0 | 0% | 2 | 2% | 3 | 3% | **0.006** | 23 | 4% |
| HPV 31 | 11 | 10% | 2 | 2% | 6 | 5% | 2 | 2% | 5 | 5% | 3 | 3% | 0.081 | 29 | 5% |
| HPV 33 | 4 | 4% | 0 | 0% | 2 | 2% | 1 | 1% | 0 | 0% | 0 | 0% | 0.083 | 7 | 1% |
| HPV 35 | 1 | 1% | 2 | 2% | 4 | 4% | 5 | 6% | 4 | 4% | 0 | 0% | 0.128 | 16 | 3% |
| HPV 39 | 6 | 6% | 3 | 3% | 4 | 4% | 1 | 1% | 3 | 3% | 6 | 7% | 0.445 | 23 | 4% |
| HPV 45 | 4 | 4% | 1 | 1% | 3 | 3% | 1 | 1% | 2 | 2% | 0 | 0% | 0.451 | 11 | 2% |
| HPV 51 | 12 | 11% | 2 | 2% | 10 | 9% | 4 | 5% | 6 | 6% | 4 | 4% | 0.096 | 38 | 6% |
| HPV 52 | 14 | 13% | 4 | 4% | 10 | 9% | 6 | 7% | 5 | 5% | 8 | 9% | 0.184 | 47 | 8% |
| HPV 56 | 3 | 3% | 2 | 2% | 7 | 6% | 1 | 1% | 3 | 3% | 1 | 1% | 0.242 | 17 | 3% |
| HPV 58 | 2 | 2% | 4 | 4% | 4 | 4% | 0 | 0% | 0 | 0% | 0 | 0% | 0.065 | 10 | 2% |
| HPV 59 | 2 | 2% | 0 | 0% | 0 | 0% | 2 | 2% | 0 | 0% | 1 | 1% | 0.251 | 5 | 1% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Any low-risk HPV | 33 | 31% | 17 | 17% | 31 | 28% | 16 | 20% | 20 | 19% | 17 | 19% | 0.106 | 134 | 23% |
| ≥ 2 low risk HPV types | 10 | 9% | 2 | 2% | 9 | 8% | 3 | 4% | 4 | 4% | 5 | 6% | 0.283 | 33 | 6% |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HPV 6 | 3 | 3% | 4 | 4% | 5 | 5% | 1 | 1% | 4 | 4% | 2 | 2% | 0.807 | 19 | 3% |
| HPV 11 | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 1 | 1% | 1 | 1% | 0.591 | 2 | 0% |
| HPV 34 | 2 | 2% | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 0.109 | 2 | 0% |
| HPV 40 | 1 | 1% | 0 | 0% | 0 | 0% | 0 | 0% | 1 | 1% | 2 | 2% | 0.358 | 4 | 1% |
| HPV 42 | 1 | 1% | 1 | 1% | 0 | 0% | 0 | 0% | 0 | 0% | 2 | 2% | 0.354 | 4 | 1% |
| HPV 43 | 5 | 5% | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 1 | 1% | **0.003** | 6 | 1% |
| HPV 44 | 3 | 3% | 0 | 0% | 2 | 2% | 1 | 1% | 3 | 3% | 3 | 3% | 0.567 | 12 | 2% |
| HPV 53 | 4 | 4% | 4 | 4% | 5 | 5% | 1 | 1% | 3 | 3% | 3 | 3% | 0.877 | 20 | 3% |
| HPV 54 | 3 | 3% | 0 | 0% | 8 | 7% | 3 | 4% | 3 | 3% | 3 | 3% | 0.122 | 20 | 3% |
| HPV 66 | 11 | 10% | 3 | 3% | 10 | 9% | 5 | 6% | 2 | 2% | 1 | 1% | **0.013** | 32 | 5% |
| HPV 68 and 73 | 2 | 2% | 2 | 2% | 4 | 4% | 1 | 1% | 2 | 2% | 2 | 2% | 0.915 | 13 | 2% |
| HPV 70 | 4 | 4% | 1 | 1% | 2 | 2% | 3 | 4% | 0 | 0% | 1 | 1% | 0.292 | 11 | 2% |
| HPV 74 | 6 | 6% | 4 | 4% | 5 | 5% | 4 | 5% | 5 | 5% | 2 | 2% | 0.916 | 26 | 4% |
|  |
| **Abbreviations:** HELIUS, HEalthy LIfe in Urban Setting; DEIA, DNA enzyme immunoassay; HPV, human papillomavirus |
| \**p* are based on Chi-squared test |  |  |  |  |  |  |  |
| *p*-values <0.05 are considered statistically significant (presented in bold) |  |  |  |  |  |  |  |
| \*\* Being tested positive for any of the 25 HPV-types that are detectable by LIPA25: 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74. |

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| **Table 3.** Association between high-risk HPV and ethnicity using logistic regression with Generalized Estimating Equations, among female HELIUS participants aged between 18 and 34 years who provided a vaginal swab and completed the questionnaire, by ethnicity, the HELIUS study Amsterdam, the Netherlands |
|
|  | **Bivariable analyses** | **Multivariable analyses** | **Multivariable analyses** |
|  | **OR** | **95% CI** | **aOR** | **95% CI** | **aOR** | **95% CI** |
|  |  |  |  |  |  |  |
| **Among 592 female participants** |  |  |  |  |  |  |
|  | **Model 1** | **Model 2** | **Model 3** |
| Dutch | 1 |  | 1 |  | **1** |  |
| South-Asian Surinamese | **0.37** | **(0.21 - 0.63)** | **0.34** | **(0.19 - 0.59)** | 0.62 | (0.36 - 1.06) |
| African Surinamese | 0.73 | (0.47 - 1.12) | **0.61** | **(0.38 - 0.96)** | 0.66 | (0.43 - 1.01) |
| Ghanaian | **0.41** | **(0.23 - 0.73)** | **0.37** | **(0.20 - 0.66)** | 0.59 | (0.33 - 1.05) |
| Moroccan | **0.49** | **(0.30 - 0.80)** | **0.47** | **(0.28 - 0.79)** | 1.46 | (0.86 - 2.50) |
| Turkish | 0.62 | (0.38 - 1.01) | 0.61 | (0.37 - 1.03) | 1.42 | (0.85 - 2.39) |
|  |  |  |  |  |  |  |
| **Among 476 female participants reporting ever having had sex with a man** |  |  |
|  | **Model 4** | **Model 5** | **Model 6** |
| Dutch | 1 |  | 1 |  | 1 |  |
| South-Asian Surinamese | **0.43** | **(0.25 - 0.75)** | **0.44** | **(0.25 - 0.78)** | 0.73 | (0.41 - 1.29) |
| African Surinamese | 0.77 | (0.50 - 1.19) | 0.67 | (0.42 - 1.07) | 0.71 | (0.45 - 1.11) |
| Ghanaian | **0.52** | **(0.29 - 0.94)** | **0.46** | **(0.24 - 0.87)** | 0.67 | (0.36 - 1.25) |
| Moroccan | **0.53** | **(0.31 - 0.93)** | 0.70 | (0.39 - 1.28) | 1.41 | (0.77 - 2.58) |
| Turkish | 0.78 | (0.48 - 1.28) | 1.00 | (0.58 - 1.72) | 1.51 | (0.86 - 2.64) |
| **Abbreviations:** HELIUS, Healthy Life in Urban Setting; HPV, human papillomavirus; STI, sexually transmitted infection; aOR, adjusted Odds Ratio; 95% CI, 95% Confidence Interval |
| Multivariable models are adjusted for the following risk factors: |
| Model 2: age (categorical), education and civil status |
| Model 3: age (categorical), education, civil status and lifetime number of male sexual partners (categorical) |
| Model 5: age (categorical), education and civil status |
| Model 6: age (categorical), education, civil status, age of sexual debut (categorical), lifetime number of male sexual partners (categorical) and sexual activity in preceding six months |